Cell Biology

REGULATED VOLUME DECREASE IN *HYLA CHRYSOSCELIS* ERYTHROCYTES.
Bridget K. Campion and Douglas B. Light*, Department of Biology, Ripon College, Ripon, WI 54971, campionb@ripon.edu, lightd@ripon.edu

Hyla chrysoscelis (Cope's gray tree frog) is one of only five freeze tolerant anurans that over winters by allowing extracellular fluid to freeze. As a result of the freezing and subsequent thawing of extracellular fluid, an osmotic imbalance with intracellular fluid is generated. Accordingly, we examined regulated volume decrease (RVD) in erythrocytes of this species. Cell volume was measured by electronic sizing with a Coulter counter (model Z2 with channelyzer). Exposure of *H. chrysoscelis* to a hypotonic (0.5X) amphibian Ringer resulted in rapid swelling of cells, followed by a slower, spontaneous RVD. Surprisingly, cells slowly swelled again approximately 15 minutes after hypotonic shock (to the best of our knowledge this behavior has not been reported for any other species). The potassium channel blocker quinine (1 M) partially inhibited RVD and completely blocked the secondary swelling phase. Substituting the impermeable cation choline for sodium in the extracellular solution not only prevented the secondary swelling, indicating it required sodium influx, but also dramatically enhanced volume recovery during that period. When the cationophore gramicidin (0.5 μM) was added to the extracellular medium it potentiated volume recovery, indicating potassium efflux is a rate-limiting step for RVD. We next examined the role of calcium in RVD. When calcium was released from intracellular stores with cyclopiazonic acid (10 μM), RVD was potentiated. However, when intracellular calcium was buffered with BAPTA (100 μM), there was no significant effect, indicating the stimulatory effect of calcium was likely an epiphenomenon. In contrast, either chelating extracellular calcium to 50 μM with EGTA or addition of the stretch-activated channel antagonist gadolinium (10 μM) blocked the secondary swelling phase, suggesting that calcium influx was required for this anomalous process. Conclusions: *H. chrysoscelis* erythrocytes respond uniquely to hypotonic shock, first exhibiting a typical RVD, which is then followed by a slower, anomalous secondary swelling. RVD depends on potassium efflux, whereas secondary swelling requires a calcium-stimulated sodium influx. (Supported by a Merck/AAAS USRP award).